"Biphasic" fevers often consist of more than two phases

ANDREJ A. ROMANOVSKY, CHRISTOPHER T. SIMONS, AND VLADIMIR A. KULCHITSKY
Thermoregulation Laboratory, Legacy Holladay Park Medical Center, Portland, Oregon 97208-3950

Romanovsky, Andrej A., Christopher T. Simons, and Vladimir A. Kulchitsky. "Biphasic" fevers often consist of more than two phases. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R323–R331, 1998.—This paper disproves the common belief that all doses of lipopolysaccharide (LPS) that are commonly referred to as biphasic fever inducing (≥2 μg/kg) cause truly biphasic responses. A catheter was implanted into the right jugular vein of several strains of adult male rats, and the animals were habituated to the experimental conditions. At an ambient temperature of 30.0°C, loosely restrained animals were injected with a 10 μg/kg dose of LPS (various preparations), and their colonic (T_c) and tail skin temperatures were monitored (from ≥1 h before to ≥7 h after the injection). The results are presented as time graphs and phase-plane plots; in the latter case the rate of change of T_c is plotted against T_c. In *experiment 1* the intravenous injection of LPS (from *Escherichia coli* 0111:B4, phenol extract) into the rats (Bkl:Wistar) induced a triphasic febrile response, as is obvious from time graphs of T_c (3 peaks), time graphs of effector activity (3 waves of tail skin vasoconstriction), and phase-plane plots (3 complete loops); the injection of saline (control) induced no T_c changes. We analyzed whether the triphasic pattern was due to some peculiarities of the experimental design, i.e., the pyrogen preparation used (*experiment 2*) or the rat strain tested (*experiment 3*) or whether this pattern reflects a more general law. In *experiment 2* we used the same (phenol) preparation of different LPS (from *Shigella flexneri* 1A and *Salmonella typhosa*) and a different preparation (TCA extract) of the same LPS (*E. coli*). Regardless of the LPS used, rats of the Bkl:Wistar strain responded to the 10 μg/kg dose with the triphasic fever. In *experiment 3*, rats of other strains [Bkl: Sprague-Dawley and Sim:(LE)fBR(Black-hooded)] were tested. Again, all animals responded to the 10 μg/kg dose of *E. coli* LPS (phenol extract) with the triphasic fever. Because all fevers caused by four different LPS preparations in three rat strains were triphasic, the triphasic pattern is likely to constitute an intrinsic characteristic of the febrile response.

febrile response; skin vasoconstriction; lipopolysaccharide preparations; rat strains; body temperature oscillations; nonlinear dynamics; phase plane

EXPERIMENTALLY, the thermoregulatory response to systemic inflammation is usually studied by inducing fever with a bolus peripheral (often intravenous) injection of a pyrogenic substance into laboratory animals (most commonly rats or rabbits). Regardless of whether the injected pyrogen is "exogenous" [such as lipopolysaccharide (LPS) and polyriboinosinic:polyribocytidilic acid (poly I:C)] or "endogenous" (such as interleukin 1, interleukin 6, and tumor necrosis factor), its injection results in a monophasic body temperature (T_b) rise if the dose of the pyrogen is low (i.e., just above threshold) but a biphasic T_b rise if the dose is several times to four orders higher. This rule has been repeatedly confirmed in a variety of species, including the rabbit (22) and the rat (29, 32).

In contrast to experimental fevers that are usually described as monophasic or biphasic, fevers observed in clinical practice are often polycyclic (46). (To accommodate the diversity of terms used in the literature, we treat the terms "phasic," "cyclic," "modal," and "peaked" interchangeably.) However, the polyphasic pattern of clinical fevers is commonly assumed to simply reflect the cyclic release of pathogens into the blood in many infections, with malaria being the best-known example of the correlation between T_b and parasitemia (18). Yet there is some anecdotal evidence (stemming from the "old" literature and the informal exchange of laboratory experience among colleagues) suggesting that a single injection of a pyrogen, even under controlled experimental conditions, can also induce a polyphasic response. Thus, "many-peaked fevers [in response to large doses of exogenous pyrogens, with] the febrile reaction being characteristically cyclic" have been described by Grant in his outstanding review (11). Similarly, the capability of poly I:C to cause triphasic fevers in rabbits has been noticed by Kimura-Takeuchi and colleagues in the laboratory of Krueger (J. M. Krueger, personal communication), even though in the published report (14) the authors did not specify the number of phases observed.

Over the past two years, our laboratory has investigated the thermoregulatory responses of ~1,000 rats to intravenous LPS in a wide dose range. A very unusual phenomenon was observed when the dose of LPS was ≥10 μg/kg. The responses of the rats to these doses appeared biphasic only if we stopped monitoring them at 3–4 h postinjection; whenever the animals were observed for longer periods (30, 33), three distinct febrile phases occurred (even if published data reflected shorter periods of time, as they did in Ref. 33).

In *experiment 1* we specifically address the issue of the number of phases in the so-called biphasic LPS-induced fever. In *experiments 2* and *3* we analyze whether the revealed febrile pattern reflects some peculiarities of the experimental design (the pyrogen preparation used, the rat strain tested) or, alternatively, is independent of these details and, rather, reflects a general law.

METHODS

Animals

Two-month-old male rats of several different strains purchased from two different sources (see *Experimental Protocols*) were used. The animals' body mass was ~200 g at the time of delivery and ~300 g at the time of experiment. The rats were initially housed three per box; after surgery they were caged singly. The room was on a 12:12-h light-dark cycle (lights on from 0700 to 1900); ambient temperature (T_a) was maintained at 22°C. Food [Teklad Rodent Diet (W) 8604,

Harlan Teklad, Madison, WI] and water were available ad libitum. The animals were handled and weighed regularly. They were also habituated (5 training sessions, 3–4 h each) to a cylindrical restrainer that limited their back-and-forth movements and prevented them from turning around; the same restrainer was used later in the experiments. All experiments were performed during the light phase (measurements started between 0800 and 0900); during this phase, rats do not actively use behavioral thermoregulation but readily recruit autonomic thermoeffector mechanisms (40). To prevent the development of tolerance to LPS, each animal was injected with LPS only once. At the end of the study the animals were killed with pentobarbital sodium (20 mg/kg iv). The protocols were approved by the Institutional Animal Care and Use Committee.

Surgical Preparation

General information. Each animal underwent two surgical operations separated by 1 wk. During the first surgery an acrylic platform was secured to the rat's skull. During the second surgery a catheter was implanted in the right jugular vein, and its exteriorized end was placed into a hollow pedestal affixed to the platform (see below). At 0.25 h before each surgery, each rat was given an antibiotic (enrofloxacin, 12 mg/kg sc) and anesthetized with ketamine-xylazine-acepromazine cocktail (55.6, 5.5, and 1.1 mg/kg ip, respectively). During each surgery the animal's body was heated by a Deltaphase Isothermal Pad (Braintree Scientific, Braintree, MA). The animal was immediately placed under a heating lamp for recovery after surgery, then it was transferred to its cage.

Surgery 1. The head of the anesthetized animal was placed into a stereotaxic instrument (model 900, David Kopf Instruments, Tujunga, CA), and a 1.5-cm-long incision was made over the sagittal suture. Subcutaneous tissues were removed by scraping. The bone was cleansed with a 3% hydrogen peroxide solution and dried with 98% ethanol. Four holes (each 0.8 mm diameter) were drilled, and four miniature stainless steel screws were screwed in the bone. The dried surface of the skull and the screws were covered with dental acrylic in such a manner that a round (~1 cm diameter) platform with a flat surface was formed. After the acrylic hardened, the rat was released from the stereotaxic instrument.

Surgery 2. The animal was placed on an operating board (Harvard Apparatus, S. Natick, MA), and a 1-cm longitudinal incision was made on the ventral surface of the neck, 1 cm to the right of the trachea. The muscles were retracted, and the right jugular vein was exposed and freed from its surrounding connective tissue. A silicone rubber catheter (0.5 mm ID, 0.9 mm OD) containing heparinized (100 U/ml) pyrogen-free saline (PFS) was passed into the superior vena cava through the jugular vein. The 15-cm free end of the catheter was pulled under the skin to the head. The wound on the ventral surface of the neck was sutured. The free end of the catheter was rolled into a coil and placed into a polypropylene vial (pedestal); the pedestal was then affixed to the platform with dental acrylic and protected with a screw-on cap. On the day after the surgery the catheter was flushed with heparinized PFS.

Instrumentation

For an experiment, each animal was instrumented with thermocouples to record temperatures of the colon (T_c; 9 cm from the anus) and tail skin (T_{sk}). The thermocouples were connected to a data logger (model AI-24, Dianachart, Rockaway, NJ) and then to a personal computer. The animal was then placed into its restrainer and transferred to a climatic chamber (Forma Scientific, Marietta, OH) set to a T_a of 30.0°C (upper limit of the thermoneutral zone for rats) and relative humidity of 50%. The exteriorized portion of the intravenous catheter was pulled through a wall port and connected to a syringe. After a 1-h stabilization period the measurements were begun, and T_c, T_{sk}, and T_a were sampled every 2 min for 8 h.

Experimental Protocols

Experiment 1. Experiment 1 was designed to determine the number of phases in the thermal response to what is thought to be a typical biphasic fever-inducing dose of LPS, i.e., 10 μg/kg. Rats of the Wistar (Bkl:Wistar) strain (B & K Universal, Kent, WA) were instrumented as described above and, 1 h after the beginning of recording, injected with LPS or PFS (1 ml/kg iv). The LPS was the phenol-extracted preparation of *Escherichia coli* 0111:B4 LPS (lot no. 35H4086, Sigma Chemical, St. Louis, MO); the same LPS is currently used by several groups working in the field (7, 16, 23). The order of the injections was alternated (i.e., one-half of the animals were injected with LPS on *day 3* and with PFS on *day 7*; one-half of the animals received PFS first and then LPS).

Experiment 2. Experiment 2 was designed to investigate whether the triphasic febrile pattern (revealed in *experiment 1*) was due to some peculiarity of the LPS preparation. On *day 3* postsurgery the animals (Bkl:Wistar) were instrumented and, 1 h after the recording was started, injected with the same dose (10 μg/kg) of the following preparations of LPS (all from Sigma Chemical): *E. coli* serotype 0111:B4, prepared by TCA extraction (lot no. 25H4008); *Shigella flexneri* serotype 1A, prepared by phenol extraction (lot no. 47F4009); and *Salmonella typhosa*, prepared by phenol extraction (lot no. 81H4018).

Experiment 3. In *experiment 3* we investigated whether the triphasic febrile pattern was due to a peculiarity of the animal strain. Rats of two other strains, Sprague-Dawley (Bkl:Sprague-Dawley, B & K Universal) and Long-Evans [Sim:(LE)fBR(Black-hooded), Simonsen Laboratories, Gilroy, CA] were tested. All tests were performed on *day 3* postsurgery. The animals were instrumented and, 1 h after the recording was started, injected with the 10 μg/kg dose of phenol-extracted *E. coli* 0111:B4 LPS.

Thermometry in Restrained Rats: A Special Consideration

Thermophysiological data obtained in restrained animals (as in the present study) are often thought to be contaminated by stress hyperthermia, a potential source of various artifacts. Such a critique is often based on the fact that the basal T_b is higher in restrained than in freely behaving rats; e.g., compare a T_c of 37.8–38.4°C reported for well-habituated, restrained male rats of the Wistar strain (31) with an intra-abdominal temperature (T_{ab}) of 37.0–37.3°C for freely moving male Wistar (24) or Sprague-Dawley (17) rats. Yet, two factors seem to explain the reported difference in T_b better than the hypothetical stress.

First, during telemetric measurements the rats are usually left in their home cages at a "room temperature" (loosely controlled at some level within 18–26°C). In contrast to this, wire thermometry requires that animals be brought to the experimental setup (usually an environmental chamber); in this case the T_a (as well as the air humidity) is tightly controlled, usually at the upper limit of thermoneutrality, i.e.,

30.0°C (8, 41). Two recent extensive studies by Gordon and Yang (9, 47) show that the T_{ab} of freely moving male rats during the day is 37.3–37.4°C at any T_a between 22.0 and 29.5°C [compare with studies by Long et al. (17) and Nakamori et al. cited above (24)], whereas if T_a increases from 29.5 to 32.0°C, the T_{ab} rises to 37.8°C [compare with our study (31)].

Second, if measured correctly (≥6.5 cm beyond the anus; see Ref. 4), T_c is one of the highest temperatures in the rat's body. It has been well documented that T_c exceeds the aortic temperature by 0.1–0.8°C (4), and it is very likely that it also exceeds T_{ab}, especially if the latter is measured near the abdominal wall. Indeed, in recent experiments, Székely (personal communication) found a difference of a few 10ths of a degree between the T_c (measured by a thermocouple) and T_{ab} (measured by a telemetric probe) in the same rat.

We conclude, therefore, that the basal T_b of restrained rats (when they are well habituated to their stocks) is comparable to that of freely moving rats and that thermometry in the restrained rat, if done correctly, remains a valuable method.

Data Processing and Analysis

To evaluate the thermal response, the absolute value of T_c was used. To evaluate the thermoeffector response of tail skin vasculature, the heat loss index (HLI) was calculated: $HLI = (T_{sk} - T_a)/(T_c - T_a)$. As explained elsewhere (28), the HLI changes between 0 (maximal vasoconstriction) and 1 (maximal dilation), with a decrease in the HLI corresponding to a decrease in the Newtonian heat loss from the tail. To compare the T_c and HLI curves between the treatments (LPS vs. PFS, *experiment 1*), a goodness-of-fit crossover test was applied: we counted the number of times the curves crossed over one another and, by summing the tail of a binomial distribution, determined the probability of the found number (or fewer) of crossovers occurring (35). To confirm the number of febrile phases found, the data were plotted in the phase-plane format [i.e., the rate of displacement change vs. the displacement; in our case the rate of T_c change, $T_c'(t)$, vs. T_c], and the number of loops (cycles) was counted. General information on this topological representation of the transient performance of a system can be found in handbooks on engineering (10); some physiological applications of the phase plane have been outlined by Partridge (26).

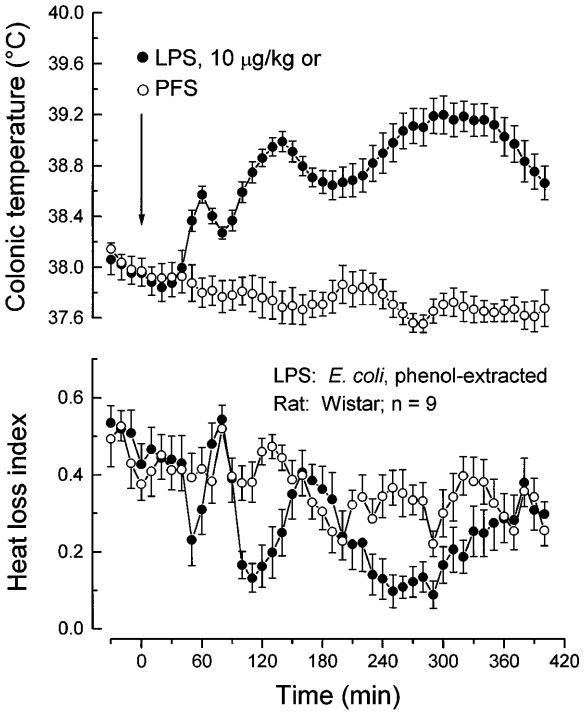

Fig. 1. Responses of Wistar (Bkl:Wistar) rats to injection (arrow) of *Escherichia coli* 0111:B4 lipopolysaccharide (LPS; phenol extract, 10 μg/kg iv) in pyrogen-free saline (PFS) and to PFS (1 ml/kg iv).

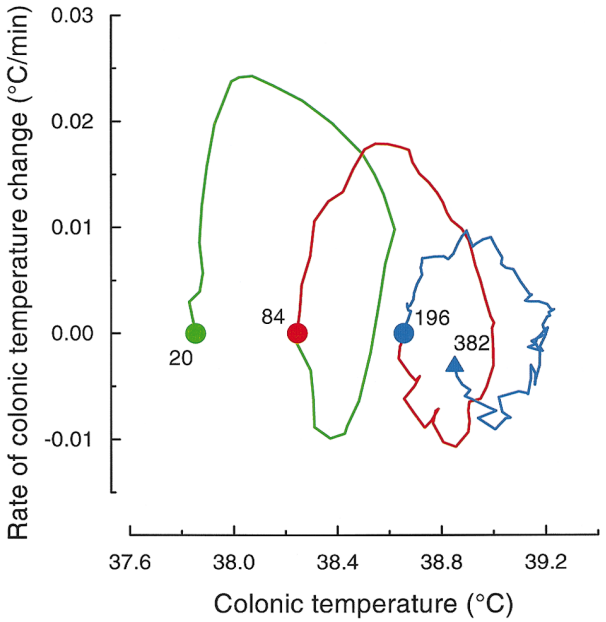

Fig. 2. Response to LPS shown in Fig. 1 plotted in phase plane. ●, Points marking beginning of each phase (chosen as points with a 0 rate of colonic temperature change and positive acceleration); number near each data point corresponds to time elapsed after injection (in minutes). Three phases can be determined in response as 3 loops of curve (cycles): *phase I* (green), from 1st circle (20 min postinjection) to 2nd circle (84 min); *phase II* (red), from 2nd circle to 3rd circle (196 min); *phase III* (blue), from 3rd circle to end of plot (▲, 382 min postinjection). For clarity, dynamics of colonic temperature during latent period of fever and during very end of experiment are not shown.

RESULTS

Experiment 1

Whereas the injection of PFS in the rats (Bkl:Wistar) did not affect their thermal state, the administration of LPS (*E. coli* 0111:B4, phenol extract; 10 μg/kg iv) induced a triphasic febrile response, with the T_c maxima at ~60, 140, and 300 min postinjection (Fig. 1, *top*). Each T_c rise was preceded by a distinct wave of tail skin vasoconstriction (a drop in HLI; Fig. 1, *bottom*). The null hypothesis (assumes that the responses to PFS and LPS are alike) was easily rejected ($P < 0.0001$) for both T_c and HLI cases. The triphasic character of the fever was confirmed by the number of cycles counted on the phase-plane plot (Fig. 2). The triphasic response appeared to be an example of damped oscillations (the magnitude of the 3rd loop is much lower than that of the 1st loop) overlapped with an overall slow shift of the system's trajectory to a higher T_c. (If the shift were subtracted, this would have transferred the plot to a concentric 3-loop coil with the loop size decreasing from outside to inside; a straight line drawn across the loops,

from inside to outside the coil, would have intersected the coil 3 times, once for each loop, or phase. The legend to Fig. 2 may be helpful in reading phase-plane plots.)

Experiment 2

Regardless of which LPS preparation was used [*E. coli*, TCA extract (Fig. 3), *Sh. flexneri*, phenol extract (Fig. 4), or *S. typhosa*, phenol extract (Fig. 5)], rats of the Bkl:Wistar strain responded to the injection of a 10 μg/kg iv dose with a triphasic T_c rise (Figs. 3*A*, 4*A*, and 5*A*, respectively, *top*). For all fevers, each rise in T_c was preceded by a separate burst of tail skin vasoconstriction (Figs. 3*A*, 4*A*, and 5*A*, *bottom*). For all fevers the triphasic pattern was confirmed in a phase-plane plot (Figs. 3*B*, 4*B*, and 5*B*).

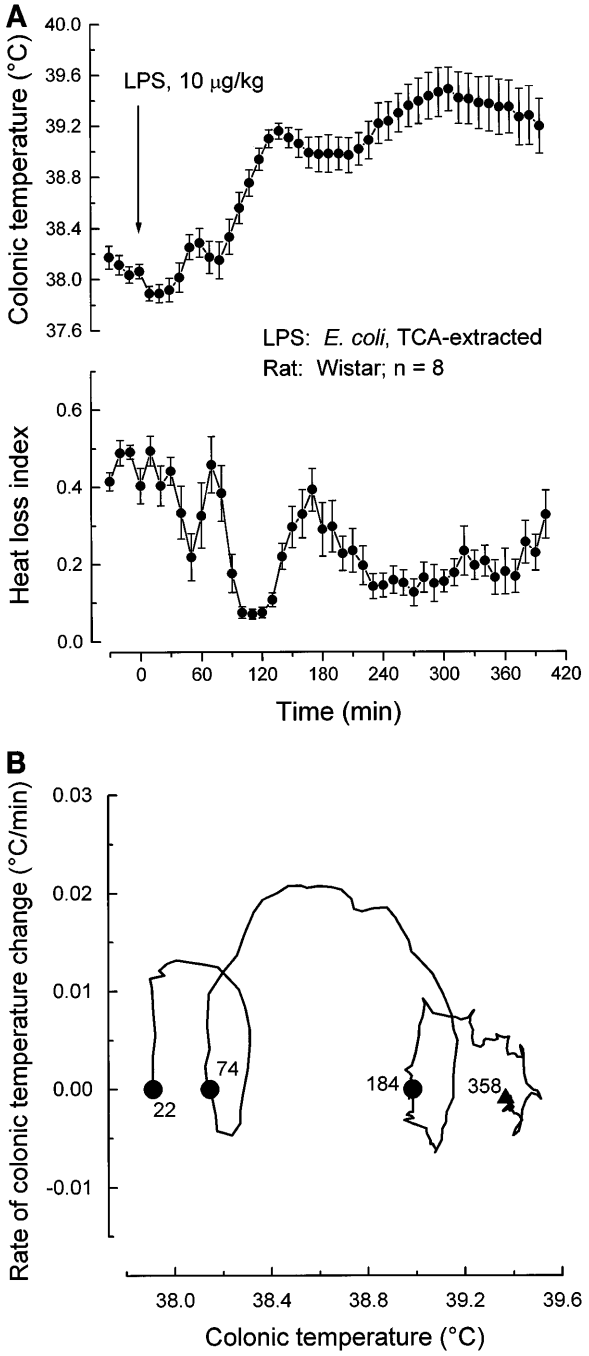

Fig. 3. Responses of Wistar (Bkl:Wistar) rats to injection (arrow) of *E. coli* 0111:B4 LPS (TCA extract, 10 μg/kg iv). See Fig. 2 legend for symbols used in phase-plane plot.

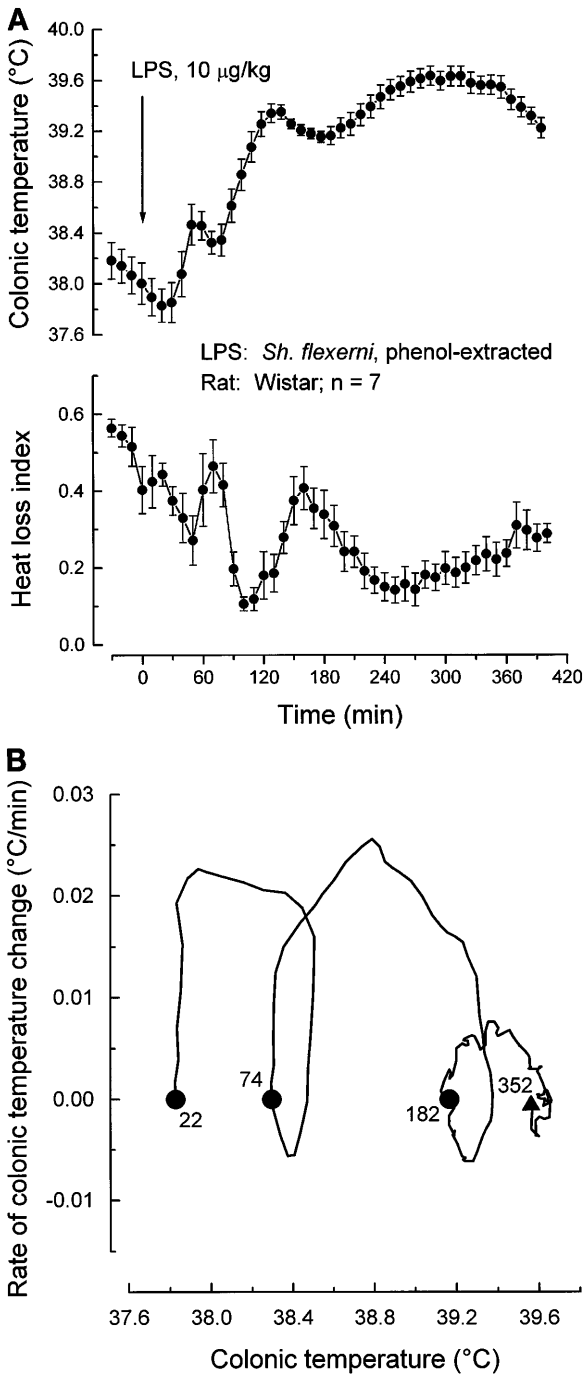

Fig. 4. Responses of Wistar (Bkl:Wistar) rats to injection (arrow) of *Shigella flexneri* 1A LPS (phenol extract, 10 μg/kg iv). See Fig. 2 legend for symbols used in phase-plane plot.

Experiment 3

Regardless of which strain of rats [Bkl:Sprague-Dawley (Fig. 6) or Sim:(LE)fBR(Black-hooded) (Fig. 7)]

was tested, the animals responded to the injection of a 10 μg/kg iv dose of *E. coli* LPS (phenol extract) with a triphasic T_c rise (Figs. 6*A* and 7*A*, respectively, *top*). For both strains, each rise in T_c was preceded by a separate burst of tail skin vasoconstriction (Figs. 6*A* and 7*A*, *bottom*). For both strains the triphasic pattern of the febrile response was confirmed in a phase-plane plot (Figs. 6*B* and 7*B*).

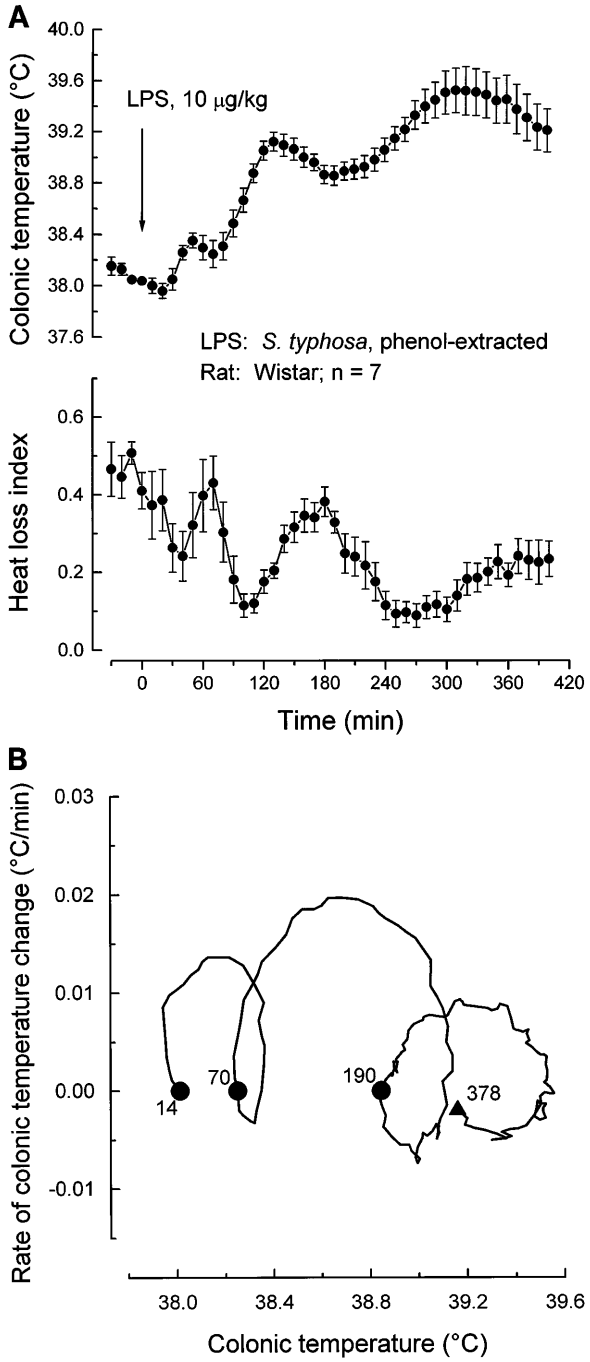

Fig. 5. Responses of Wistar (Bkl:Wistar) rats to injection (arrow) of *Salmonella typhosa* LPS (phenol extract, 10 μg/kg iv). See Fig. 2 legend for symbols used in phase-plane plot.

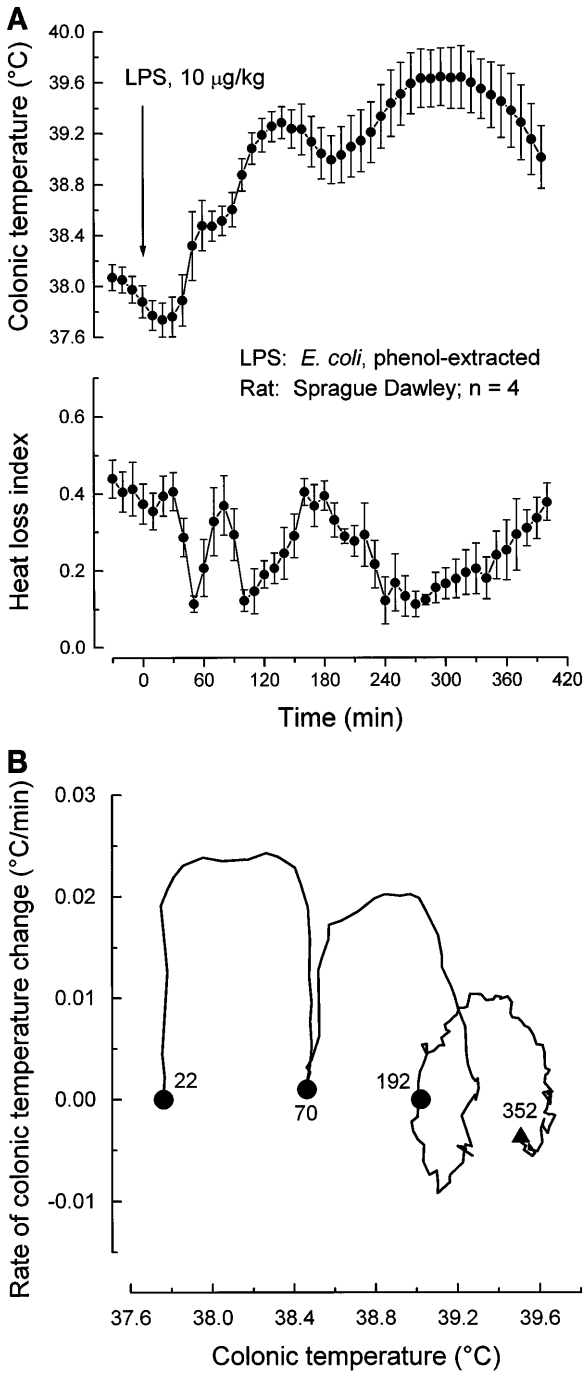

Fig. 6. Responses of Sprague-Dawley (Bkl:Sprague-Dawley) rats to injection (arrow) of *E. coli* 0111:B4 LPS (phenol extract, 10 μg/kg iv). See Fig. 2 legend for symbols used in phase-plane plot.

DISCUSSION

Triphasic Fever

Within the last decade, quite a few studies on fever have been dedicated to identifying the separate triggers and differentiating the biochemical/physiological portraits of the two febrile phases (19–21, 27, 29, 34, 42, 44). The methodological issues related to distinguishing between monophasic and biphasic responses have

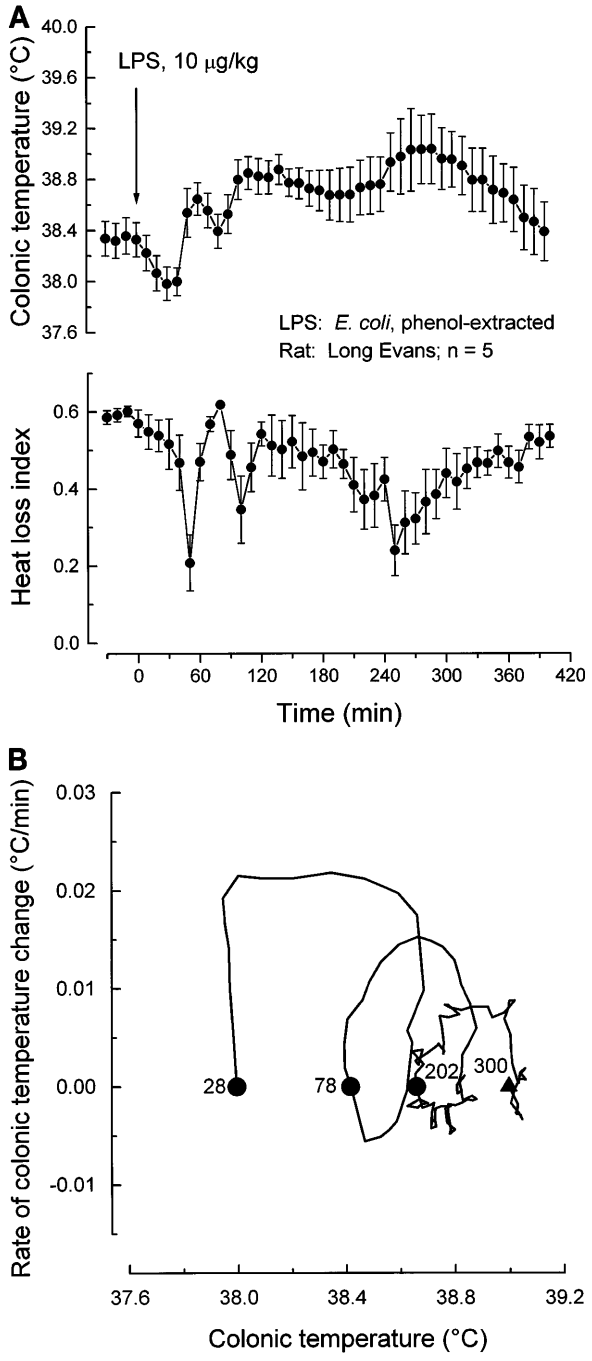

Fig. 7. Responses of Long-Evans [Sim:(LE)fBR(Black-hooded)] rats to injection (arrow) of *E. coli* 0111:B4 LPS (phenol extract, 10 μg/kg iv). See Fig. 2 legend for symbols used in phase-plane plot.

also been subjected to an in-depth analysis (15). In addition, the specific roles of febrile *phases I* and *II* in the adaptation to infection have been discussed (29, 34). Despite such keen attention, the phenomenon of the bimodality remains an unsolved mystery: not only is there little agreement on the details of the underlying machinery (for review see Ref. 27), but, as the present report shows, even the number of phases in the "biphasic" fever might have been miscounted.

The results of *experiment 1* demonstrate that the febrile response of the rat strain studied (Bkl:Wistar) to the preparation of LPS used (*E. coli* 0111 B:4, phenol extract) in the dose chosen (~10 times higher than the monophasic fever-inducing dose) consisted of three phases. Each phase was characterized by a distinct T_c rise (with the maxima at ~60, 140, and 300 min postinjection), a distinct burst of effector activity (tail skin vasoconstriction), and a separate loop on a phase plane [$T_c'(t)$ vs. T_c]. None of the three phases could have been explained by the experimental procedure per se or by the circadian rhythm of T_c, because no febrile phases occurred when the injection of LPS was replaced with the injection of the same volume of PFS. It is reasonable to suggest, therefore, that the triphasic response is an authentic response of the animals tested to the LPS injected, but is this triphasic pattern characteristic for this particular rat strain (tested in *experiment 1*) only, for the preparation of LPS (used in this experiment) only, or, alternatively, for LPS fever in the rat in general?

LPS Preparations

Numerous LPS preparations (extracted from different bacteria and by different procedures) are commercially available. The difference in these preparations has been repeatedly blamed for contributing to the great variability of physiological responses to LPS within the same species or even strain (12). Wan and Grimble (45) reported that LPS from *E. coli* 0127:B8 prepared by butanol extraction induced fever in rats, whereas a TCA extract of the same LPS (as well as of LPS from the 055:B5 serotype of *E. coli*) caused hypothermia. Horan and coauthors (12) found differences of two orders among the potencies of three different LPS preparations (from *E. coli* serotype 0127:B8 prepared by TCA extraction, *E. coli* 0127:B8 prepared by phenol extraction, and *E. coli* 026:B6 prepared by TCA extraction) in inducing fever (and its underlying thermogenic response) in the rat; the phenol extract was ranked the most potent.

We asked whether the triphasic febrile pattern observed in the present work might have been specific for the pyrogen tested, either its bacterial source (*E. coli* serotype 0111:B4) or the procedure used for its preparation (phenol extraction). However, *experiment 2* rejected such a proposition: regardless of the source of LPS (*E. coli* 0111:B4, *Sh. flexneri* 1A, or *S. typhosa*) and the extraction procedure (based on phenol or TCA), the febrile pattern was always triphasic. Furthermore, all preparations tested produced quantitatively comparable responses. It can be excluded, therefore, that the triphasic pattern is just a peculiar attribute of the febrile response to one particular LPS preparation.

Rat Strains

Another possibility we considered was that the triphasic fevers observed in *experiments 1* and *2* could have reflected just a specific feature of the rat strain used,

i.e., Wistar. This possibility was definitely worth exploring, because the rat's thermoregulation appears to possess some strain specificity. Thus, as reviewed by Gordon (8), the "normal" level of T_b, the magnitude of its diurnal change, the preferred T_a, the limit of heat tolerance, the resistance to hypothermia, the mass of brown adipose tissue, and several other traits often differ distinctly between such strains as genetically obese and lean, hypertensive and normotensive, albino (e.g., Wistar or Sprague-Dawley) and pigmented (e.g., Long-Evans). Albino rats are less active, have a lower T_b, and prefer a higher T_a than their pigmented counterparts (8). Albino strains are also distinct in many other (nonthermoregulatory) physiological and behavioral aspects (2). It would not be surprising at all if the febrile responsiveness varies within the rat species, at least to some extent.

An example of such variability has been provided by studies of the Brattleboro strain (genetically deficient in arginine vasopressin; suffers from diabetes insipidus). The responsiveness of Brattleboro rats to a peripheral administration of LPS has been repeatedly found to differ from that of the parental strain, Long-Evans, and another commonly used strain, Sprague-Dawley (5, 13, 25, 39, 43). In work by Stitt and Shimada (39), Brattleboro rats responded with fever to the dose of LPS that caused hypothermia in the Sprague-Dawley rats. [To make the picture more complete, it should be added, however, that the 5 cited reports are mutually contradictory (for a critical analysis, see Ref. 39) and that a 6th study (37) failed to reveal any peculiarities in the febrile responsiveness of the Brattleboro strain.] Even more intriguing data have been obtained by comparison between genetically lean and genetically obese Zucker rats. The two are not only known to respond differently to pyrogenic stimuli (3) but, moreover, have been recently shown to respond to the same dose of LPS by developing a different number of febrile phases (36).

In *experiment 3* we tested animals of pigmented, i.e., Long-Evans [Sim:(LE)fBR(Black-hooded)], and albino strains, i.e., Wistar (Bkl:Wistar) and Sprague-Dawley (Bkl:Sprague-Dawley); moreover, the animals were purchased from two different companies. Despite this heterogeneity, all the animals uniformly responded to intravenous LPS with a triphasic febrile response. Furthermore, while this study was in preparation, our pilot experiments (n = 3; unpublished observations) with another Wistar strain [Sim:(WI)fBR] showed that these rats similarly developed triphasic fevers in response to *E. coli* 0111:B4 LPS (phenol preparation, 10-μg/kg iv). The present study suggests, therefore, that the triphasic febrile pattern is not just a peculiarity of a certain rat strain. Yet, to be objective, we should add that our study did not include several potentially interesting rat strains, such as Brattleboro and Zucker (see above), Lewis (possesses a wide range of endocrine and immune abnormalities) (38), and FOK (heat resistant) (6).

Concluding Remark

It is important to add that we do not reject the well-established ability of some doses of LPS to induce biphasic fevers in several species, including the rat. If the dose of LPS is within a narrow range of ~2–8 μg/kg (i.e., higher than the monophasic fever-inducing dose but lower than 10 times that), the febrile response of the rat may be truly biphasic. We do dispute, however, the belief that all doses of LPS that are commonly referred to as biphasic fever-inducing doses (10–10,000 μg/kg) cause only two T_b peaks. The present paper and its companion (30) disprove this belief by showing that higher doses of LPS induce not biphasic but triphasic responses. Because all fevers caused by different LPS preparations in three different rat strains were triphasic, we suggest that the triphasic pattern constitutes an intrinsic characteristic of the febrile response. The fact that a single bolus injection of a pyrogen may result in several T_b rises is likely to change our ideas on the nature of polycyclic, multipeaked fevers normally occurring in natural diseases. Interestingly, Carlson et al. (1) recently showed that the febrile response of rats to live intravenously administered *E. coli* is indistinguishable from that to LPS; both responses closely resemble the triphasic fevers described here.

Perspectives

> All I could see from where I stood
> Was three long mountains and a wood.
>
> E. St. V. Millay, "Renascence"

This study immediately raises two questions. The first question is whether febrile *phase III* is the last one. What would have happened if the rats tested in the present experiments had been injected with a higher dose of LPS and/or observed for a longer period of time? Is it possible that we would have seen, in Millay's words, more "mountains" behind the puzzling "wood"? We do not have an answer to this question, but we think that the more general term "polyphasic" (or "polycyclic") may be preferred to the specific "triphasic" for describing fevers with more than two T_b peaks.

The second question is why, over the last few decades, everyone in the field [including ourselves (27, 29)] seemed consistently to see only "two mountains," whereas, from where we are presently standing, "three long mountains" are not only visible but are strikingly obvious? This question is answered in the companion paper (30).

Discussions on the phase plane with Dr. L. D. Partridge and statistical consultations with Dr. L. D. Homer are gratefully acknowledged. Dr. J. M. Macpherson kindly reviewed an early draft of the manuscript and provided important feedback. The authors also thank N. V. Romanovsky for indispensable help with the animal care and J. Emerson-Cobb for excellent editorial assistance.

V. A. Kulchitsky was on leave from the Institute of Physiology, Minsk 220072, Belarus.

This study was made possible by intramural support and a research grant-in-aid from Legacy Health System (Portland, OR).

Address for reprint requests: A. A. Romanovsky, Thermoregulation Laboratory, Legacy Holladay Park Medical Center, PO Box 3950, Portland, OR 97208-3950.

Received 14 July 1997; accepted in final form 18 March 1998.

REFERENCES

1. **Carlson, D. E., J. K. Babus, N. Nguyuza, H. Melhem-Stancofski, and B. J. Eastridge.** Role of endotoxin in the response to experimentally induced bacteremia in chronically prepared rats. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1562–R1570, 1997.
2. **Creel, D.** Inappropriate use of albino animals as models in research. *Pharmacol. Biochem. Behav.* 12: 969–977, 1980.
3. **Dascombe, M. J., A. Hardwick, R. A. Lefeuvre, and N. J. Rothwell.** Impaired effects of interleukin-1β on fever and thermogenesis in genetically obese rats. *Int. J. Obes.* 13: 367–373, 1989.
4. **Donhoffer, S.** *Homeothermia of the Brain (Cerebral Blood Flow, Metabolic Rate, and Brain Temperature in the Cold: the Possible Role of Neuroglia).* Budapest, Hungary: Akadémiai Kiadó, 1980.
5. **Eagan, P. C., N. W. Kasting, W. L. Veale, and K. E. Cooper.** Absence of endotoxin fever but not prostaglandin E_2 fever in the Brattleboro rat. *Am. J. Physiol.* 242 (*Regulatory Integrative Comp. Physiol.* 11): R116–R120, 1982.
6. **Furuyama, F., and K. Ohara.** Genetic development of an inbred rat strain with increased resistance adaptation to a hot environment. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R957–R962, 1993.
7. **Goldbach, J.-M., J. Roth, B. Störr, and E. Zeisberger.** Repeated infusions of TNF-α cause attenuation of the thermal response and influence LPS fever in guinea pigs. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R749–R754, 1996.
8. **Gordon, C. J.** *Temperature Regulation in Laboratory Rodents.* Cambridge, UK: Cambridge University Press, 1993.
9. **Gordon, C. J., and Y. Yang.** Contribution of spontaneous motor activity to the 24 hour control of body temperature in male and female rats. *J. Therm. Biol.* 22: 59–68, 1997.
10. **Graham, D., and D. McRuer.** The phase plane method. In: *Analysis of Nonlinear Control Systems.* New York: Dover, 1961, chapt. 7, p. 272–314.
11. **Grant, R.** Physiological effects of heat and cold. *Annu. Rev. Physiol.* 13: 75–98, 1951.
12. **Horan, M. A., R. A. Little, N. J. Rothwell, and P. J. L. M. Strijbos.** Comparison of the effects of several endotoxin preparations on body temperature and metabolic rate in the rat. *Can. J. Physiol. Pharmacol.* 67: 1011–1014, 1989.
13. **Kandasamy, S. B., and B. A. Williams.** Absence of endotoxin-fever but not hyperthermia in Brattleboro rats. *Experientia* 39: 1343–1344, 1983.
14. **Kimura-Takeuchi, M., J. A. Majde, L. A. Toth, and J. M. Krueger.** The role of double-stranded RNA in induction of the acute-phase response in an abortive influenza virus infection model. *J. Infect. Dis.* 166: 1266–1275, 1992.
15. **Kluger, M. J.** Fever: role of pyrogens and cryogens. *Physiol. Rev.* 71: 93–127, 1991.
16. **LeMay, L. G., A. J. Vander, and M. J. Kluger.** Role of interleukin 6 in fever in rats. *Am. J. Physiol.* 258 (*Regulatory Integrative Comp. Physiol.* 27): R798–R803, 1990.
17. **Long, N. C., S. L. Kunkel, A. J. Vander, and M. J. Kluger.** Antiserum against tumor necrosis factor enhances lipopolysaccharide fever in rats. *Am. J. Physiol.* 258 (*Regulatory Integrative Comp. Physiol.* 27): R332–R337, 1990.
18. **Miller, L. H.** Transfusion malaria. In: *Transmissible Diseases and Blood Transfusion,* edited by T. J. Greenwalt and C. A. Jamieson. New York: Grune & Stratton, 1974, p. 241–266.
19. **Morimoto, A., N. Murakami, T. Myogin, M. Takada, S. Teshirogi, and T. Watanabe.** Separate mechanisms inside and outside the blood-brain barrier inducing metabolic changes in febrile rabbits. *J. Physiol. (Lond.)* 392: 637–649, 1987.
20. **Morimoto, A., N. Murakami, T. Nakamori, and T. Watanabe.** Evidence for separate mechanisms of induction of biphasic fever inside and outside the blood-brain barrier in rabbits. *J. Physiol. (Lond.)* 383: 627–637, 1987.
21. **Morimoto, A., N. Murakami, T. Nakamori, and T. Watanabe.** Multiple control of fever production in the central nervous system of rabbits. *J. Physiol. (Lond.)* 397: 269–280, 1988.
22. **Morimoto, A., T. Nakamori, T. Watanabe, T. Ono, and N. Murakami.** Pattern differences in experimental fevers induced by endotoxin, endogenous pyrogen, and prostaglandins. *Am. J. Physiol.* 254 (*Regulatory Integrative Comp. Physiol.* 23): R633–R640, 1988.
23. **Morrow, L. E., J. L. McClellan, J. J. Klir, and M. J. Kluger.** The CNS site of glucocorticoid negative feedback during LPS- and psychological stress-induced fevers. *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R732–R737, 1996.
24. **Nakamori, T., A. Morimoto, K. Morimoto, N. Tan, and N. Murakami.** Effects of α- and β-adrenergic antagonists on rise in body temperature induced by psychological stress in rats. *Am. J. Physiol.* 264 (*Regulatory Integrative Comp. Physiol.* 33): R156–R161, 1993.
25. **Ngsee, J., C. J. Jenkins, and N. Wilson.** Temperature response to endotoxin in Brattleboro rats (Abstract). *Physiol. Can.* 11: 109, 1980.
26. **Partridge, L. D.** Physiology is not an appendage of anatomy—illustrated with deterministic chaos. *Korean J. Physiol.* 26: 1–13, 1992.
27. **Romanovsky, A. A., and C. M. Blatteis.** Biphasic fever: what triggers the second temperature rise? *Am. J. Physiol.* 269 (*Regulatory Integrative Comp. Physiol.* 38): R280–R286, 1995.
28. **Romanovsky, A. A., and C. M. Blatteis.** Heat stroke: opioid-mediated mechanisms. *J. Appl. Physiol.* 81: 25–65, 1996.
29. **Romanovsky, A. A., V. A. Kulchitsky, N. V. Akulich, S. V. Koulchitsky, C. T. Simons, D. I. Sessler, and V. N. Gourine.** First and second phases of biphasic fever: two sequential stages of the sickness syndrome? *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R244–R253, 1996.
30. **Romanovsky, A. A., V. A. Kulchitsky, C. T. Simons, and N. Sugimoto.** Methodology of fever research: why are polyphasic fevers often thought to be biphasic? *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R332–R338, 1998.
31. **Romanovsky, A. A., V. A. Kulchitsky, C. T. Simons, N. Sugimoto, and M. Székely.** Cold defense mechanisms in vagotomized rats. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R784–R789, 1997.
32. **Romanovsky, A. A., O. Shido, S. Sakurada, N. Sugimoto, and T. Nagasaka.** Endotoxin shock: thermoregulatory mechanisms. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R693–R703, 1996.
33. **Romanovsky, A. A., C. T. Simons, M. Székely, and V. A. Kulchitsky.** The vagus nerve in the thermoregulatory response to systemic inflammation. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R407–R413, 1997.
34. **Romanovsky, A. A., and M. Székely.** Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Med. Hypotheses* 50: 219–226, 1998.
35. **Rosen, D.** *Mathematics Recovered for the Natural and Medical Sciences.* London: Chapman & Hall, 1992.
36. **Rosenthal, M., J. Roth, B. Störr, and E. Zeisberger.** Fever response in lean (*Fa/−*) and obese (*fa/fa*) Zucker rats and its lack to repeated injections of LPS. *Physiol. Behav.* 59: 787–793, 1996.
37. **Ruwe, W. D., W. L. Veale, and K. E. Cooper.** Characterization of the febrile response in the Brattleboro rat. In: *Environment, Drugs and Thermoregulation,* edited by P. Lomax and E. Schönbaum. Basel: Karger, 1983, p. 128–134.
38. **Sternberg, E. M., J. M. Hill, G. P. Chorousos, T. Kamilaris, S. J. Listwak, P. W. Gold, and R. L. Wilder.** Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. *Proc. Natl. Acad. Sci. USA* 86: 2374–2378, 1989.
39. **Stitt, J. T., and S. G. Shimada.** A comparison of the febrile responses of the Brattleboro and Sprague-Dawley strains of rats to endotoxin and endogenous pyrogens. *Can. J. Physiol. Pharmacol.* 65: 1377–1381, 1987.
40. **Sugimoto, N., O. Shido, S. Sakurada, and T. Nagasaka.** Day-night variations of behavioral and autonomic thermoregula-

tory responses to lipopolysaccharide in rats. *Jpn. J. Physiol.* 46: 451–456, 1996.
41. **Szymusiak, R., and E. Satinoff.** Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate. *Physiol. Behav.* 26: 687–690, 1981.
42. **Tøien, Ø., and J. B. Mercer.** Effect of total body core cooling during poly I:C-induced fever in rabbits. *Am. J. Physiol.* 268 (*Regulatory Integrative Comp. Physiol.* 37): R1257–R1265, 1995.
43. **Veale, W. L., P. C. Eagan, and K. E. Cooper.** Abnormality of the febrile response of the Brattleboro rat. *Ann. NY Acad. Sci.* 394: 776–779, 1982.
44. **Vybíral, S., L. Černý, and L. Janský.** Mode of ACTH antipyretic action. *Brain Res. Bull.* 21: 557–562, 1988.
45. **Wan, J., and R. F. Grimble.** Diurnal influences of the metabolic effects of two types of *Escherichia coli* endotoxin in rats (Abstract). *Proc. Nutr. Soc.* 45: 83A, 1986.
46. **Woodward, T. E.** The fever pattern as a clinical diagnostic aid. In: *Fever: Basic Mechanisms and Management*, edited by P. A. Mackowiak. New York: Raven, 1991, p. 83–103.
47. **Yang, Y., and C. J. Gordon.** Ambient temperature limits and stability of temperature regulation in telemetered male and female rats. *J. Therm. Biol.* 21: 353–363, 1996.